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Workshop on Cytokines and Parasites

Final Report

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19. ABSTRACT (Continue on reverse if necessary and identify by block number) Immunoregulatory cytokines are the hormonal factors that direct and fine-tune effector reactions of immune responses. Spectacular advances in technologies for isolation and purification of these cytokines, in particular the development of monoclonal antibody and molecular biology methodologies, have enabled scientists to examine the regulatory roles of single cytokines, as well as analyze the interrelationships and synergy between different cytokines. The application of this information to immunotherapy of neoplastic diseases has proceeded beyond the animal model stage, and several different cytokines are presently in clinical trials (Interleukin-2, Granulocyte-macrophage Colony Stimulating Factors, Interferon alpha). The one day meeting on Parasites and Cytokines, held in conjunction with the International Congress of the Reticuloendothelial Society (Kauai, Hawaii, October 17-27, 1987) focused attention of the immunological community on the unique problems associated with parasitic diseases, and explored the options of immunotherapy in infectious diseases for which there are presently no vaccines, or for which direct stimulation of the host's immune system during infection might be detrimental to survival.					
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Immunoregulatory cytokines are the hormonal factors that direct and fine-tune effector reactions of immune responses. Spectacular advances in technologies for isolation and purification of these cytokines, in particular the development of monoclonal antibody and molecular biology methodologies, have enabled scientists to examine the regulatory roles of single cytokines, as well as analyze the interrelationships and synergy between different cytokines. The application of this information to immunotherapy of neoplastic diseases has proceeded beyond the animal model stage, and several different cytokines are presently in clinical trials (Interleukin-2, Granulocyte-macrophage Colony Stimulating Factors, Interferon alpha). Yet the concept of immunotherapy in infectious diseases has received only marginal attention. The one day meeting on Parasites and Cytokines, held in conjunction with the International Congress of the Reticuloendothelial Society (Kauai, Hawaii, October 17-21, 1987) focused attention of the immunological community on the unique problems associated with parasitic diseases, and explored the options of immunotherapy in infectious diseases for which there are presently no vaccines, or for which direct stimulation of the host's immune system during infection might be detrimental to survival.

Attending the workshop were 45 invited participants and members of the Reticuloendothelial Society. The workshop was divided into six interrelated sessions: Cytokine Regulation of Microbial Attachment/Ingestion by Cells (Discussion Leader, David Sacks, NIH, Bethesda, MD); Cytokine Regulation of Macrophage Antimicrobial Activities (Discussion Leader, Priscilla Campbell, National Jewish Hospital, Denver, CO); Cytokine Effects on nonRES Cells (Discussion Leader, Gerald Byrne, University of Wisconsin, Madison, WI); Effector Cytokines with Anti-microbial Activity (Discussion Leader, Carol Nacy, Walter Reed Army Institute of Research, Washington, DC); Parasite Regulation of

Cytokine Production During Infections (Discussion Leader, John Mansfield, University of Wisconsin, Madison, WI); and In Vivo Effects of Cytokines in Infectious Diseases (Discussion Leader, Hannah Shear, New York University, New York, NY). Each Discussion Session was approximately 1 hr in length, and involved several 5-10 minute presentations by selected members of the audience and a general Discussion of the topic led by the Discussion Leader.

Discussion in the session on Cytokine Regulation of Parasite Attachment or Ingestion centered on the role of complement (C') in facilitating attachment of organisms to macrophages. Dr. David Mosser (Cornell University) showed data that all species of Leishmania fix C', and that promastigotes (the insect form of this parasite) actually survive better intracellularly if coated with C3. This C' coat is mobile, and can be regulated by the parasite. The major glycoprotein on the surface of promastigotes, gp63, may actually clip the alpha chain of C3b to produce C3bi, thus regulating which C' receptor, CR1 (C3b) or CR3 (C3bi), the parasite uses for entry. Molecules that enter by CR1 do not trigger a respiratory burst. Dr. David Sacks (NIH) continued the discussion by demonstrating that infective promastigotes have phenotypic characteristics quite distinct from those of noninfective parasites. Infective organisms have different surface carbohydrates (peanut agglutinin, PNA, negative), fail to trigger a respiratory burst, fix C' by the classical pathway, are relatively resistant to C'-mediated lysis, and bind C' noncovalently; noninfective promastigotes are PNA positive, trigger a respiratory burst, and fix C' by the alternate pathway. Infective promastigotes use CR1 for attachment to macrophages, thus providing an explanation for the lack of respiratory burst during phagocytosis of these parasites. Dr. Marcus Horwitz (UCLA) described the unusual phagocytic mechanism that Legionella pneumophila uses to enter macrophages: a rolling, or coiling of the bacter-

tum. These organisms also fix C' by the alternate pathway, and use one of the C' receptors for entry. The C' acceptor molecule on the bacterium has been isolated, and is a 30-40 kD major outer membrane protein.

In the session on Cytokine Regulation of Macrophage Antimicrobial Activities, Dr. Priscilla Campbell (National Jewish Hospital) demonstrated the dichotomy of macrophage killing functions with Listeria and tumor cells. Inflammatory cells with no tumoricidal activity, but good bactericidal activity, can be activated by gamma interferon (IFN) to kill tumor cells, but lose bactericidal activity in the process. Dr. Henry Murray (Cornell) emphasized the major role that IFN plays as an activation factor for intracellular destruction of several parasites, and discussed tryptophan depletion as one mechanism by which IFN-activated macrophages restrict replication of some, but not all, obligately intracellular parasites. Several other cytokines (TNF, GM-CSF, CSF-1) are not effective for induction of macrophage killing of Toxoplasma and Leishmania donovani. Dr. Carol Nacy (Walter Reed) pointed out that lymphokines depleted of IFN have residual activity for induction of macrophage intracellular killing of Leishmania, and that these IFN-depleted lymphokines also contain a factor that suppresses IFN activation of macrophages for killing of Leishmania, without suppressing IFN-induced macrophage Ia antigen expression. The role of IFN may be fine-tuned by factors that direct IFN activity towards induction of immune reactions rather than effector reactions. NonIFN macrophage activation factors that can trigger killing of parasites may also work in specific tissue compartments in the absence of IFN. Dr. Steven Reed (Biomedical Research Institute, Seattle, WA) demonstrated that GM-CSF, greater than 500 U/ml, induced the intracellular destruction of Trypanosoma cruzi by macrophages whether the cells were treated pre- or postinfection. GM-CSF also induced a modest respiratory burst at the time of

parasite attachment to treated cells. He speculated that GM-CSF may be the first cytokine the macrophage encounters in the inflammation associated with parasite inoculation, prior to the antigen-specific release of IFN. As the immune response progresses, there may be a synergistic interaction between GM-CSF and IFN, since GM-CSF appears to restore the immune responsiveness of isolated spleen cells from infected animals. Dr. Albert Zlotnick (DNAX) mentioned that IL-4 increases the uptake of Trypanosoma cruzi by macrophages, and induces a modest killing of the intracellular parasite.

Dr. Gerald Byrne (University of Wisconsin) opened the session Cytokine Effects on nonKES Cells by describing the interaction of IFN with Chlamydia-infected fibroblasts: IFN increases the uptake and degradation of tryptophan by fibroblasts, which in turn suppresses the replication the chlamydial reticulate body (RB). IFN treatment does not, however, affect the intracellular transformation of the infectious EB particle to the replicative RB. Dr. Schofield (NYU) described the marked intracellular destruction of malaria sporozoites induced by treatment of hepatocytes with IFN. Dr. Nacy (Walter Reed) suggested that there may be additional factors in human lymphokines or obtained from T cell lines that affect intracellular Plasmodium falciparum sporozoite survival in an established hepatoma cell line. The discussion diverged at this time to include two brief talks on novel cells that secrete lymphokines. Dr. Michail Sitkovsky (NIH) described the surprisingly rapid production of IFN by cytotoxic T cells. Once the T cell receptor is triggered, it takes less than 2 hr for this cell to produce greater than 800 U/ml IFN. This IFN is not preformed in the cytosol or packaged in the characteristic cytotoxic T cell granules, but is synthesized de novo. In contrast, Dr. Arnold Greenburg (University of Manitoba) presented evidence for a preformed granule-associated macrophage activation factor in large granular lymphocytes

that is not IFN or any of the other characterized macrophage activation factors.

The discussion on Effector Cytokines began with Dr. Marcus Horwitz (UCLA), who concentrated on iron deprivation as an effector mechanism of activated microbicidal macrophages. He presented data that IFN downregulated transferrin receptors on macrophages, thereby decreasing iron transport into the cell. As Legionella requires iron for replication, he suggested that iron starvation may be the effector mechanism that IFN-activated macrophages use for limiting growth of this bacterium. Dr. Patricia Kongshavn (McGill University) initiated a discussion of the role of tumor necrosis factor (TNF) in parasitic diseases by describing an exceedingly complex interaction of TNF and Trypanosoma musculi. TNF administered to mice early during disease actually enhanced parasite growth, but had a modest antitrypanosomal effect later in the infection. In vitro, TNF exerted a slight antitrypanosomal effect against isolated parasites, but had no effect in the presence of cells. In fact, under these conditions, TNF enhanced the replication of T. musculi. Dr. Miodrag Belosevic (Walter Reed) reported that TNF had no effect on isolated Leishmania major amastigotes, but could induce macrophage resistance to infection, an activated macrophage effector reaction that decreases the uptake of this obligate intracellular parasite. The activity of TNF for induction of this macrophage function was dramatically increased in the presence of IFN, a cytokine that cannot induce the reaction by itself. Dr. Schofield (NYU) reiterated the synergistic interactions of TNF and IFN for intracellular destruction of Plasmodia, and presented data that administration of antibodies to IFN γ substantially reduces protection of animals immunized against malaria infection. Interestingly, antiIFN antibodies do not affect the course of infection in naive animals. Dr. Greg Bancroft (Washington University) men-

tioned that results with Listeria were quite different: administration of antiIFN antibodies in this model resulted in lethal disease, while control animals receiving irrelevant antibodies resolved their infections.

Dr. John Mansfield (University of Wisconsin) began the session on Parasite Effects on Cytokine Production with a discussion of the African trypanosomes. Although Trypanosoma rhodesiense replicates primarily in blood, it can be ingested by macrophages. Trypanosome-infected macrophages do not, however, present antigen in a context that can be interpreted by T lymphocytes. Consequently, T cells fail to produce IL-2, and fail to proliferate. This abnormal antigen presentation is not a consequence of the downregulation of Ia antigen expression on the infected macrophage, nor is it a consequence of decreased IL-1 production by infected macrophages. In fact, Dr. Bancroft (Washington University) presented evidence for an abnormally high level of circulating IL-1 during peak parasitemia with another of the african trypanosomes, T. brucei. The function of this IL-1, and whether it is beneficial or detrimental to the host, is unknown. Dr. Neil Reiner (University of British Columbia) found that macrophages infected with Leishmania donovani, unlike those infected with the african trypanosomes, fail to express Ia antigen, and fail to produce IL-1 in the presence of IFN. Infected cells have the same number of IFN receptors as uninfected macrophages, and the intracellular events that transmit the signal for Ia antigen expression are intact. By some unknown mechanism, intracellular leishmania depress the production of Ia antigen mRNA. Pretreatment of the cells with IFN for a minimum of 4 hr before infection can, however, enhance L. donovani amastigotes as a signal for IL-1 production. Dr. Arisha Ilczak presented data that IL-2 production was dramatically decreased in H2 noncure C57Bl/6 congenic mice infected with L. donovani. This cytokine defect could be overcome by irradiating the mice with 550

Rads prior to infection, suggesting a cellular basis for the lack of circulating IL-2.

The final session of the workshop, In Vivo Effects of Cytokines, was opened by Dr. Campbell (National Jewish Hospital). She presented data that IFN has chemotactic properties. As a macrophage activation agent for intracellular destruction of Listeria, however, it was less than optimal: rather than kill the bacterium, IFN merely induced cytostasis of Listeria in infected cells. She suggested that depletion of IFN in vivo with antiIFN antibodies affected primarily the induction of an immune response by decreasing Ia antigen expression. Dr. Byrne (University of Wisconsin) demonstrated that the administration of 10^6 U of IFN one day before and the day of challenge cured mice of lethal chlamydial pneumonia. Dr. Anne Fortier (Walter Reed) used the macrophage activation agent BCG to induce nonspecific protection against both cutaneous infection and systemic disease in mice infected with a lethal strain of Leishmania major. A biproduct of this nonspecific protection was the development of specific immunity to rechallenge with leishmania, a unique adjuvant property of macrophage activation agents. Dr. Somesh Sharma (Applied Immunosciences) discussed the protective effects of IL-2 administration on experimental toxoplasmosis. Three to five hundred Units IL-2 protected L3T4 lymphocyte-depleted mice from lethal infection, and decreased the number of Toxoplasma gondi cysts from over 10,000 in control mice to 200-300 cysts in treated mice. Dr. Murray (Cornell) described the imminent human clinical trials of IFN and AZT in AIDS patients. He also mentioned the increase in hepatic parasites in mice infected with Leishmania donovani that were treated before and during infection with anti-IFN antibodies. Dr. Hannah Shear (NYU) discussed the paradoxical effect of IFN administration on the resistance of mice to lethal and nonlethal Plamsodium yoelii infections. Injection of 1000-

100,000 U IFN per day protected both inbred (BALB/ByJ) and outbred (SW) mice from malaria induced by the lethal strain of plasmodium (17xL), but had little, if any effect on infections with the nonlethal strain. Moreover, in naturally resistant mice such as CBA/J, IFN had only a slight effect on the course of 17xL P. yoelii and no effect at all on the nonlethal parasite. This paradox was not resolved in discussion. The final presentation of the workshop was given by Dr. Peter Krammer (West Germany) who summarized the role of macrophage activation in resistance to Schistosoma infections.

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